



PATENT
Serial No. 08/418,870
Attorney Docket No. 0085.006

40.
D. Williams
6/25/97

I hereby certify that this paper is being deposited in the United States Postal Service as first class mail in an envelope addressed to the Assistant Commissioner of Patents, Washington, D.C. 20231-0001 on May 14, 1997.

Barbara G. McClung 5/14/97
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Gary Van Nest, et al.
Serial No.: 08/418,870 Group: 1813
Filed: April 7, 1995 Examiner: H. Auer
For: ADJUVANT FORMULATION COMPRISING
SUBMICRON OIL DROPLET EMULSION

DECLARATION UNDER 37 C.F.R. 1.132

Assistant Commissioner of Patents
Washington, D.C. 20231-0001

Sir:

1. We, Gary A. Van Nest, of 4890 San Pablo Road, El Sobrante, California; Gary Ott, of 112 Marlow Drive, Oakland, California; and Gail L. Barchfeld, of 2225 Romey Lane, Hayward, California, do swear that we are co-inventors of the above-captioned patent application, Serial No. 08/418,870.

Consented
5/18/97

2. In our laboratories, we performed the experiments summarized in paragraphs 3, 4, and 5 below. These data clearly demonstrate that our submicron oil-in-water adjuvant compositions can have a potent adjuvant activity even when delivered to a site remote from the site of antigen delivery. This adjuvant activity could not be associated with any antigen depot effect.

3. Materials and Methods: Groups of 10 New Zealand White rabbits were used. One group of animals was injected with 25 µg of recombinant gD2 from herpes simplex virus (HSV) without adjuvant in the thigh muscle. A second group of rabbits was injected almost simultaneously with 25 µg of gD2 without adjuvant in one thigh and with 0.25 ml of "MF59" adjuvant (a submicron oil-in-water adjuvant composition having 5% squalene (v/v), 0.5% polysorbitan 80, 0.5% sorbitan trioleate, in citrate buffer) in the opposite thigh. Booster immunizations identical to the primary immunizations were given 21 days later. 14 days after each immunization, animals were bled and anti-gD2 antibody titers were determined by enzyme linked immunoadsorbant assay.

4. The antibody results are shown:

Group	Rabbit Number	Anti-gD2 titer 14 days post 1 st	Anti-gd2 titer 14 days post 2nd
1 gD2 without adjuvant	497	31	847
	498	16	1524
	499	5	67
	500	23	600
	501	17	126
	502	33	1310
	503	21	43
	504	500	963
	505	13	320
	506	13	51
	geometric mean ± standard error	24 ± 9	300 ± 133
2 gD2 in one thigh MF59 in opposite thigh	487	34	9216
	488	27	4001
	489	595	29115
	490	44	5868
	491	97	8173
	492	35	4636
	493	88	6797
	494	12	1004
	495	583	4433
	496	17	546
	geometric mean ± standard error	62 ± 26	4596 ± 1229

5. Conclusions: After one immunization, MF59 delivered in the opposite thigh was able to stimulate antibody titers to gD2 approximately three-fold. After two immunizations, MF59 delivered in the opposite thigh stimulated titer approximately 15-fold.

6. We declare that all statements made herein of our knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereof.

Date:

May 14, 1997

Gary A. Van Nest

Date:

May 14, 1997

Gary Ott

Date:

May 14, 1997

Gail L. Barchfeld